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11/22/00

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Akio Miyao

For : A NOVEL GENE INVOLVED IN BRASSINOSTEROID
RESPONSES

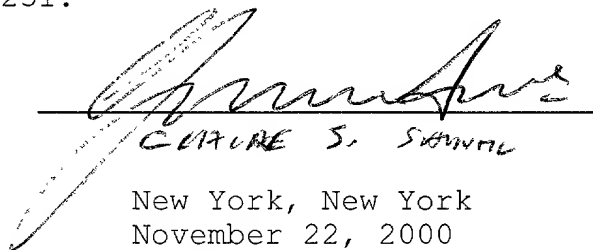
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Hon. Commissioner
for Patents
Washington, D.C. 20231
Box PATENT APPLICATION


CATHERINE S. SAMML
New York, New York
November 22, 2000

TRANSMITTAL LETTER FOR
ORIGINAL PATENT APPLICATION

Sir:

Transmitted herewith for filing are the
[X] specification; [X] claims; [X] abstract; [X] declaration
and [X] power of attorney, for the above-identified patent
application.

Also transmitted herewith are:

[X] 7 sheets of:

[X] Formal drawings.

[] Informal drawings. Formal drawings will be filed
during the pendency of this application.

☐ Certified copy(ies) of application(s)

(country)	(appln. no.)	(filed)
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(country)	(appln. no.)	(filed)

from which priority is claimed.

☐ An assignment of the invention to _____.

☐ A check in the amount of \$40.00 to cover the recording fee.

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FOR	NUMBER FILED	NUMBER EXTRA	RATE	FEE
BASIC FEE				\$710.00
TOTAL CLAIMS	3	- 20 = 0	X \$ 18 =	\$ 0.00
INDEPENDENT CLAIMS	1	- 3 = 0	X \$ 80 =	\$ 0.00
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIMS			+ \$270 =	\$ 0.00
TOTAL				<u>\$710.00</u>

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[] This application is being filed unaccompanied by a filing fee. The appropriate filing fee will be paid in response to a Notice to File Missing Parts, pursuant to 37 C.F.R. § 1.53(f).

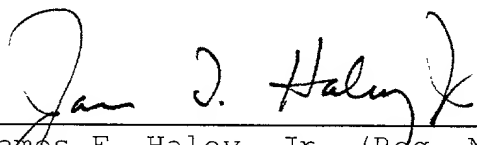
[X] The Commissioner is hereby authorized to charge payment of any additional filing fees required under 37 C.F.R. § 1.16, in connection with the paper(s) transmitted herewith, or credit any overpayment of same, to deposit Account No. 06-1075. A duplicate copy of this transmittal letter is transmitted herewith.

[] Amend the specification by inserting before the first line the sentence: -- This is a [] continuation-in-part, of application Serial No.: _____, filed

entitled _____

_____.

[] Please charge \$_____ to Deposit Account No. 06-1075 in payment of the filing fee. A duplicate copy of this transmittal letter is transmitted herewith.


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APPLICATION INFORMATION

Title Line One:: A NOVEL GENE INVOLVED IN BRASSINOSTEROID
Title Line Two:: RESPONSES
Total Drawing Sheets:: 7
Formal Drawings?: Yes
Application Type:: Utility
Docket Number:: MAFF-1
Secrecy Order in Parent Appl.?: No

REPRESENTATIVE INFORMATION

Representative Customer Number:: 1473
Registration Number One:: 27794

PRIOR FOREIGN APPLICATIONS

Foreign Application One:: 2000-149106
Filing Date:: 05-19-2000
Country:: Japan
Priority Claimed:: Yes

Source:: PrintEFS Version 1.0.1

AR022

A NOVEL GENE INVOLVED IN BRASSINOSTEROID RESPONSES

EI124987820US

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION:

5 The present invention relates to a novel gene. In particular, the present invention relates to a novel gene in plants which encodes a protein having the function of controlling an in-vivo signal transduction system in a physiological reaction system against brassinosteroid
10 hormone.

2. DESCRIPTION OF THE RELATED ART:

15 Transposons are mutagenic genes which are known to be ubiquitous in animal, yeast, bacterial, and plant genomes. Transposons are classified into two classes, Class I and Class II, depending on their transposition mechanisms. Transposons belonging to Class II are transposed in the form of DNAs without being replicated. Known Class II
20 transposons include the Ac/Ds, Spm/dSpm and Mu elements of Zea mays (Fedoroff, 1989, Cell 56, 181-191; Fedoroff et al., 1983, Cell 35, 235-242; Schiefelbein et al., 1985, Proc. Natl. Acad. Sci. USA 82, 4783-4787), and the Tam element of Antirrhinum majus (Bonas et al., 1984, EMBO J., 3,
25 1015-1019). Class II transposons are widely used for gene isolation techniques which utilize transposon tagging. Such techniques utilize the fact that a transposon induces physiological and morphological changes when inserted into genes. The affected gene can be isolated by detecting such
30 changes (Bancroft et al., 1993, The Plant Cell, 5, 631-638; Colasanti et al., 1998, Cell, 93, 593-603; Gray et al., 1997, Cell, 89, 25-31; Keddie et al., 1998, The Plant Cell, 10, 877-887; Whitham et al., 1994, Cell, 78, 1101-1115).

Transposons belonging to Class I, also referred to as retrotransposons, are replicated and transposed via RNA intermediates. Class I transposons were first identified and characterized in *Drosophila* and in yeasts. However, recent studies have revealed that Class I transposons are ubiquitous in plant genomes and account for a substantial portion of the genomes (Bennetzen, 1996, *Trends Microbiolo.*, 4, 347-353; Voytas, 1996, *Science*, 274, 737-738). A large majority of retrotransposons appear to be inactive. Recent studies indicate that some of these retrotransposons are activated under stress conditions such as injuries, pathogenic attacks, or cell culture (Grandbastien, 1998, *Trends in Plant Science*, 3, 181-187; Wessler, 1996, *Curr. Biol.* 6, 959-961; Wessler et al., 1995, *Curr. Opin. Genet. Devel.* 5, 814-821). Activation under stress conditions has been reported for *Tnt1A* and *Tto1* in tobacco (Pouteau et al., 1994, *Plant J.*, 5, 535-542; Takeda et al., 1988, *Plant Mol. Biol.*, 36, 365-376), and *Tos17* in rice (Hirochika et al., 1996, *Proc. Natl. Acad. Sci. USA*, 93, 7783-7788), for example.

The *Tos17* retrotransposon of rice is one of the most-extensively studied plant Class I elements in plants. *Tos17* was cloned by an RT-PCR method using a degenerate primer prepared based on a conservative amino acid sequence in reverse transcription enzyme domains between *Tyl-copia* retroelements (Hirochika et al., 1992, *Mol. Gen. Genet.*, 233, 209-216). *Tos17* is 4.3kb long, and has two 138bp LTRs (long chain terminal repetitions) and PBS (primer binding sites) complementary to the 3' end of the start methionine tRNA (Hirochika et al., 1996, *supra*). *Tos17* transcription is strongly activated through tissue culture, and its copy

number increases with culture time. In Nipponbare, a model Japonica cultivar used for genome analysis, two copies of Tos17 are initially present, which are increased to 5 to 30 copies in a regenerated plant after tissue culture (Hirochika et al., 1996, supra). Unlike Class II transposons which were characterized in yeasts and Drosophila, Tos17 is transposed in chromosomes in random manners and causes stable mutation, and therefore provides a powerful tool for functional analysis of rice genes (Hirochika, 1997, Plant Mol. Biol. 35, 231-240; 1999, Molecular Biology of Rice (ed. by K. Shimamoto, Springer-Verlag, 43-58).

SUMMARY OF THE INVENTION

The present invention relates to a polynucleotide encoding a plant gene capable of controlling a signal transduction system for brassinosteroid hormone, the polynucleotide encoding an amino acid sequence from Met at position 1 to Arg at position 1057 of SEQ ID NO: 2 in the SEQUENCE LISTING, including any polynucleotide encoding an amino acid sequence in which one or more amino acids are deleted, substituted or added to the amino acid sequence.

In one embodiment of the invention, the polynucleotide may be derived from rice.

In another embodiment of the invention, the polynucleotide may be as represented by SEQ ID NO: 1 in the SEQUENCE LISTING.

The present invention further relates to methods for controlling various effects in plants in which

brassinosteroid hormone is involved, e.g., growth promotion, yield increase, quality improvement, maturation enhancement, and tolerance against biotic and abiotic stresses.

5

The inventors diligently conducted systematic analyses of phenotypes of plants having a newly transposed Tos17 copy and sequences adjoining Tos17 target sites with respect to rice. As a result, the inventors found a dwarf rice mutation obtained from Tos17 insertion, and isolated the gene responsible for this mutation by utilizing Tos17 as a tag, thereby accomplishing the present invention.

10

Thus, the invention described herein makes possible the advantage of providing a novel plant gene which can be provided by using Tos17.

15

This and other advantages of the present invention will become apparent to those skilled in the art upon reading and understanding the following detailed description with reference to the accompanying figures.

20

BRIEF DESCRIPTION OF THE DRAWINGS

25

Figures 1A and 1B are photographs showing a brassinosteroid non-sensitive mutant having Tos17 inserted therein, which was found among regenerated Akitakomachi lineage. On the left of each figure is shown a brassinosteroid non-sensitive mutant having Tos17 inserted therein. On the right of each figure is shown a wild type plant body. Figure 1A evidences an influence toward dwarfism and upright form. Figure 1B evidences an influence toward malformation of grain hulls.

30

Figures 2A and 2B are photographs showing a brassinosteroid non-sensitive mutant having Tos17 inserted therein, which was found among regenerated Nipponbare lineage. On the left of each figure is shown a brassinosteroid non-sensitive mutant having Tos17 inserted therein. On the right of each figure is shown a wild type plant body. Figure 2A evidences an influence toward dwarfism and upright form. Figure 2B evidences an influence toward malformation of grain hulls.

Figure 3A shows a Northern analysis autoradiogram of RNA extracted from the leaves of a brassinosteroid non-sensitive mutant (Akitakomachi) and RNA extracted from various organs of a wild type rice plant (Nipponbare).

Figure 3B shows a Northern analysis autoradiogram of RNA extracted from brassinosteroid non-sensitive mutants and RNA extracted from wild type rice plants. The left-hand side of Figure 3B shows a comparison between wild types and mutants obtained by using a 5' probe. The right-hand side of Figure 3B shows a comparison between wild types and mutants obtained by using a 3' probe.

Figure 4 shows an amino acid sequence of the novel rice gene which controls a physiological reaction system induced by brassinosteroid hormone, together with characteristic sequences found therein (where nuclear localization signals and an ATP/GTP binding motif can be observed).

Figure 5A shows a brassinosteroid leaf blade bending experimentation using a mutated line (A0369) derived

from Akitakomachi. The left-hand side shows results of wild type plants, whereas the right-hand side shows results of mutants.

5 Figure 5B shows a brassinosteroid leaf blade bending experimentation using a mutated line (NC6148) derived from Nipponbare. The left-hand side shows results of wild type plants, whereas the right-hand side shows results of mutants.

10 DESCRIPTION OF THE PREFERRED EMBODIMENTS

 The present invention provides a method for producing an improved plant, the method utilizing a novel
15 plant gene which can be provided by using Tos17.

 According to the present invention, there is provided a polynucleotide encoding a plant gene capable of controlling various effects in which brassinosteroid
20 hormone is involved. As used herein, the term "capable of controlling various effects" means the ability to control various effects in plants in which brassinosteroid hormone is involved, e.g., growth promotion, yield increase, quality improvement, maturation enhancement, and tolerance against
25 biotic and abiotic stresses, including dwarfism, upright form, and malformation of grain hulls, thereby providing a number of agriculturally useful effects as are attained by treatments with brassinosteroid hormone agricultural chemicals. The term "plants" encompasses both
30 monocotyledons and dicotyledons.

 A polynucleotide encoding a plant gene capable of controlling a signal transduction system for

brassinosteroid hormone according to the present invention is, for example, a polynucleotide encoding an amino acid sequence from Met at position 1 to Arg at position 1057 of SEQ ID NO: 2 in the SEQUENCE LISTING, including any
5 polynucleotide encoding an amino acid sequence in which one or more amino acids are deleted, substituted or added to the aforementioned amino acid sequence.

10 A polynucleotide encoding a plant gene capable of controlling various effects in which brassinosteroid hormone is involved encompasses any polynucleotides which have at least about 80% sequence homology, preferably at least about 85% sequence homology, and more preferably at least about 90% sequence homology, still more preferably
15 at least about 95% sequence homology, and most preferably at least about 99% sequence homology, with an amino acid sequence from Met at position 1 to Arg at position 1057 of SEQ ID NO: 2 in the SEQUENCE LISTING, so long as they are capable of controlling various effects in plants in which
20 brassinosteroid hormone is involved. The term "sequence homology" indicates a degree of identity between two polynucleotide sequences to be compared with each other. The rate (%) of sequence homology between two polynucleotide sequences for comparison is calculated by, after optimally
25 aligning the two polynucleotide sequences for comparison, obtaining a matched position number indicating the number of positions at which identical ("matched") nucleic acid bases (e.g., A, T, C, G, U, or I) are present in both sequences, dividing the matched position number by total number of bases
30 in the polynucleotide sequences for comparison, and multiplying the quotient by 100. The sequence homology can be calculated by using the following sequencing tools, for example: a Unix base program designated GCG Wisconsin

Package (Program Manual for the Wisconsin Package, Version 8, September 1994, Genetics Computer Group, 575 Science Drive Madison, Wisconsin, USA 53711; Rice, P. (1996) Program Manual for EGCG Package, Peter Rice, The Sanger Centre, Hinxton Hall, Cambridge, CB10 1RQ, England), and the ExPASy World Wide Web molecular biology server (Geneva University Hospital and University of Geneva, Geneva, Switzerland).

Cells into which genes have been introduced are first selected based on drug resistance, e.g., hygromycin resistance, and then regenerated into plant bodies by using usual methods.

The terminology and laboratory procedures described throughout the present specification are directed to those which are well-known and commonly employed in the art. Standard techniques may be used for recombination methods, polynucleotide synthesis, microorganisms culturing, and transformation (e.g., electroporation). Such techniques and procedures are generally known from various standard textbooks available in the field or by way of the present specification (including a generally-referenced textbook by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). Such literature is incorporated herein by reference.

The polynucleotide according to the present invention can be obtained by using the method described herein, for example. However, the polynucleotide according to the present invention may also be obtained by any chemical synthesis process based on the sequence disclosed herein. For example, the polynucleotide according to the present

invention may be synthesized by using a polynucleotide synthesizer available from Applied Bio Systems in accordance with the instructions provided by the manufacturer.

5 Methods of PCR amplification are well-known in the art (PCR Technology: Principles and Applications for DNA Amplification, ed. HA Erlich, Freeman Press, NewYork, NY (1992); PCR Protocols: A Guide to Methods and Applications, Innis, Gelfland, Snisky, and White, Academic Press, San
10 Diego, CA(1990); Mattila et al. (1991) Nucleic Acids Res. 19: 4967; Eckert, K.A. and Kunkel, T. A. (1991) PCR Methods and Applications 1: 17; PCR, McPherson, Quirk, and Taylor, IRL Press, Oxford). Such literature is incorporated herein by reference.

15

(Examples)

 Hereinafter, the present invention will be described by way of examples which are of illustrative but non-limiting nature.

20

(Example 1: Activation of Tos17 via culture)

 Using fully ripened seeds of Nipponbare and Akitakomachi, which are varieties of Japonica subspecies, induction of calli and cell suspension culture were carried
25 out as described earlier (Hirochika et al., 1996, supra). The activation of Tos17 which was used for gene destruction was carried out following the method of Ohtsuki (1990) (rice protoplast culture system, Food and Agricultural Research Development Association). In summary, fully ripened seeds
30 of rice were cultured in an MS medium having 2,4-dichlorophenoxyacetic acid (2,4-D) added thereto (2 mg/ml) (Ohtsuki (1990), supra) (25°C, 1 month), to induce callus formation. The resultant calluses were cultured for

5 months in an N6 liquid medium having 2,4-D added thereto (Ohtsuki (1990), supra), and thereafter placed on a redifferentiation medium (Ohtsuki (1990), supra), whereby redifferentiated rice plants were obtained (first generation (R1) plants).

(Example 2: Isolation of sequences adjoining Tos17)

Utilizing each of the regenerated R1 rice plants obtained according to Example 1 as a first strain, about 1000 R1 seeds were collected from each strain and grown on a paddy field to obtain second generation (R2) plants, which were subjected to a morphological analysis. As a result of observing the phenotypes of the respective plant bodies in the R2 group, it was learned that about 1/4 of the R2 group of an Akitakomachi strain A0369 exhibit the "dwarfism, upright form, and malformation of grain hulls" phenotype (Figures 1A and 1B). In the regenerated group of Akitakomachi, dwarfism, upright form, and malformation of grain hulls were observed for brassinosteroid insensitive mutants (Figure 1A, left, and Figure 1B, left), as compared with the wild type (Figure 1A, right, and Figure 1B, right). The isolation of adjacent sequences of transposed Tos17, which is co-segregating with the phenotypes, was carried out by an IPCR method (Ochman et al., Genetics Nov; 120(3): 621-3(1988) and Triglia et al., Nucleic Acids Res Aug 25; (16): 8186(1988)). The total DNA of A0369 was digested with XbaI, and a ligation process was performed in a large quantity of solution, thereby obtaining self-ligated circular molecules. In the self-ligated circular molecules, the adjacent sequences are flanking the internal sequence of Tos17. As a result, amplification was successfully carried out by usual PCR methods using an outward primer pair (T17TAIL3: GAGAGCATCATCGGTTACATCTTCTC; T17-1950R:

TCTAGCAGTCTCAATGATGTGGCG) based on the known sequence of Tos17.

(Example 3: Search for alleles)

5 Using the sequence obtained according to Example 2,
lineage in which Tos17 had been inserted at a different site
in the same gene was selected by PCR from the regenerated
rice group of Nipponbare. As a result, a line (NC6148) which
similarly exhibited dwarfism, upright form, and
10 malformation of grain hulls were observed for
brassinosteroid was selected. That is, in the regenerated
rice group of Nipponbare, as well, dwarfism, upright form,
and malformation of grain hulls were observed for
brassinosteroid insensitive mutants (Figure 2A, left, and
15 Figure 2B, left), relative to the wild type (Figure 2A,
right, and Figure 2B, right). It was concluded that these
common mutations were results of the same gene having been
destroyed.

20 (Example 4: Expression and analysis of the causative gene)

From the group of R2 rice plants (selfed progeny from
the A0369 and NC6148 strains) obtained according to
Examples 2 and 3, individuals exhibiting mutation were
identified from normal individuals. RNA was prepared from
25 both groups of individuals, and the expression specificity
was analyzed through Northern analysis. After agarose
electrophoresis, the RNA obtained from individuals
exhibiting mutation and the RNA obtained from normal
individuals were allowed to adsorb to nylon membranes. DNA
30 fragments which were obtained by amplifying via PCR a
sequence (positions 5775-6638 of the genomic sequence) on
the 5' side and a sequence (positions 8175-8765 of the
genomic sequence) on the 3' side of the Tos17 insertion site

in both mutated lines were labeled with ^{32}P -dCTP. By using these as probes, expression specificity was analyzed through Northern analysis (Figures 3A and 3B). As seen from the Northern analysis autoradiogram shown in Figure 3A, a band (about 4.3 kb) indicated by an arrow was confirmed to be expressed in all observed organs of the wild type. However, in the mutants, transcripts of abnormal sizes were observed due to the insertion of Tos17, indicating that the natural function of the wild type is lost (Figure 3B).

(Example 5: Structural analysis of the causative gene)

Using the sequence obtained according to Example 2 as a probe, the corresponding cDNA and genomic clone were obtained from a cDNA library and a genomic library. Their structures are shown in SEQ ID Nos: 1 and 3. It was learned that this gene includes 6 exons and 5 introns, encoding 1057 amino acids, and that Tos17 had been inserted at the 4th and 5th exons in two mutants, respectively. Moreover, motif search results suggested the presence of nuclear localization signal 1 (amino acid residues 329-367 of SEQ ID NO: 2, Robbins & Dingwall consensus sequence; a search result by PSORT program (<http://psort.ims.u-tokyo.ac.jp/>)) and nuclear localization signal 2 (amino acid residues 457-460, 595-600 of SEQ ID NO: 2, 4 amino acid nuclear localization pattern signal; a search result by PSORT program (<http://psort.ims.u-tokyo.ac.jp/>)) as well as the presence of an ATP/GTP binding domain (amino acid residues 526-533 of SEQ ID NO: 2; a search result by a motif search service on Genomenet(<http://www.genome.ad.jp/>)). Thus, the possibility of this gene being involved in signal transduction was suggested (Figure 4).

(Example 6: brassinosteroid sensitivity evaluation)

5 The present gene was deduced to be a factor involved
in the signal transduction system for plant hormones, taking
note of the facts that the present gene was expressed in
all plant bodies and that pleiotropic influences resulted
from destroying this gene, as well as the possibility that
the gene might be a factor involved in the signal
transduction system. Presuming that the signal
transduction system is that for brassinosteroid hormone in
view of the resultant upright form, the inventors performed
10 a leaf blade bend response test as a brassinosteroid response
test, by using brassinolide, which is one kind of
brassinosteroid hormone. The second leaf of rice which was
allowed to germinate in the dark was cut off, and immersed
in a 1 ng/ml of brassinosteroid solution for 48 hours. The
15 wild type individuals having the wild type genes showed
bending of the leaf blades and leaf sheath junctions
(left-hand side in Figures 5A and 5B), showing response to
brassinolide, whereas mutant individuals showed little
bending thereof (right-hand side in Figures 5A and 5B),
20 indicating that the destruction of the present gene resulted
in the loss of response to brassinosteroid. From the above
results, it was revealed that the present gene is a gene
involved in the signal transduction system for
brassinosteroid hormone.

25 The above examples are illustrative, and by no means
limiting, of various aspects of the present invention and
the manners in which the oligonucleotide according to the
present invention can be made and utilized.

30 Thus, according to the present invention, a novel
polynucleotide is provided which is capable of controlling
various effects in which brassinosteroid hormone is involved,

the polynucleotide being of use in plant breeding. By introducing the present polynucleotide into plants and artificially controlling various effects in which brassinosteroid hormone is involved, it is expected that effects such as growth promotion, yield increase, quality improvement, maturation enhancement, and tolerance against biotic and abiotic stresses can be controlled, thereby providing a number of agriculturally useful effects as are attained by treatments with brassinosteroid hormone agricultural chemicals.

Various other modifications will be apparent to and can be readily made by those skilled in the art without departing from the scope and spirit of this invention. Accordingly, it is not intended that the scope of the claims appended hereto be limited to the description as set forth herein, but rather that the claims be broadly construed.

SEQUENCE LISTING

<110> National Institute of Agrobiological Resources, Ministry of Agriculture,
Forestry and Fisheries

5 Japan Science and Technology Corporation

<120> A novel gene involved in brassinosteroid responses

<130>

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<140> JP

<141> 2000-05-22

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<170> PatentIn Ver. 2.1

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cgaccgacca cggcgaccag tcgatctccc tctccgggcg ccaaccgcgt cttagcttca 240

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1

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5 10 15

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25 Cys Met Leu Ala Arg Gly Gly Thr Gly Ala Val Ala Pro Val Leu Glu

20 25 30

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Phe Gln Ser Thr Gln Glu Gln Gln Thr His Leu Arg Met Glu Glu Met
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	Ser Ile Ala Gln Ser Trp Thr Arg Gly Lys Gly Lys Met Val His Pro			
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1. A polynucleotide encoding a plant gene capable of controlling a signal transduction system for brassinosteroid hormone, the polynucleotide encoding an amino acid sequence from Met at position 1 to Arg at position 1057 of SEQ ID NO: 2 in the SEQUENCE LISTING, including any polynucleotide encoding an amino acid sequence in which one or more amino acids are deleted, substituted or added to the amino acid sequence.

3. A polynucleotide according to claim 1 as represented by SEQ ID NO: 1 in the SEQUENCE LISTING.

There is provided a polynucleotide encoding a plant gene capable of controlling a signal transduction system for brassinosteroid hormone, the polynucleotide encoding an amino acid sequence from Met at position 1 to Arg at position 1057 of SEQ ID NO: 2 in the SEQUENCE LISTING, including any polynucleotide encoding an amino acid sequence in which one or more amino acids are deleted, substituted or added to the amino acid sequence.

A **Mutant** **Wild Type**
 (A0369 -/-) (Akitakomachi)



B **Mutant** **Wild Type**
 (A0369 -/-) (Akitakomachi)



Fig. 1

[illegible]

Fig. 2

Nipponbare Wild Type		Akitakomachi Wild Type (leaf)	
seedling		seedling	
root		root	
leaf		leaf	
callus		callus	

seedling
ro

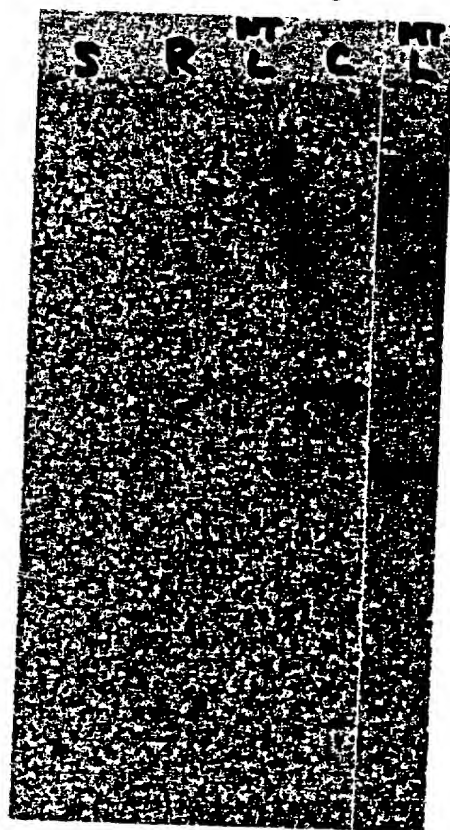
root

leaf

callus.

Akitakomachi
Wild Type

Wild Type (leaf)



Wild Type band

Fig. 3A

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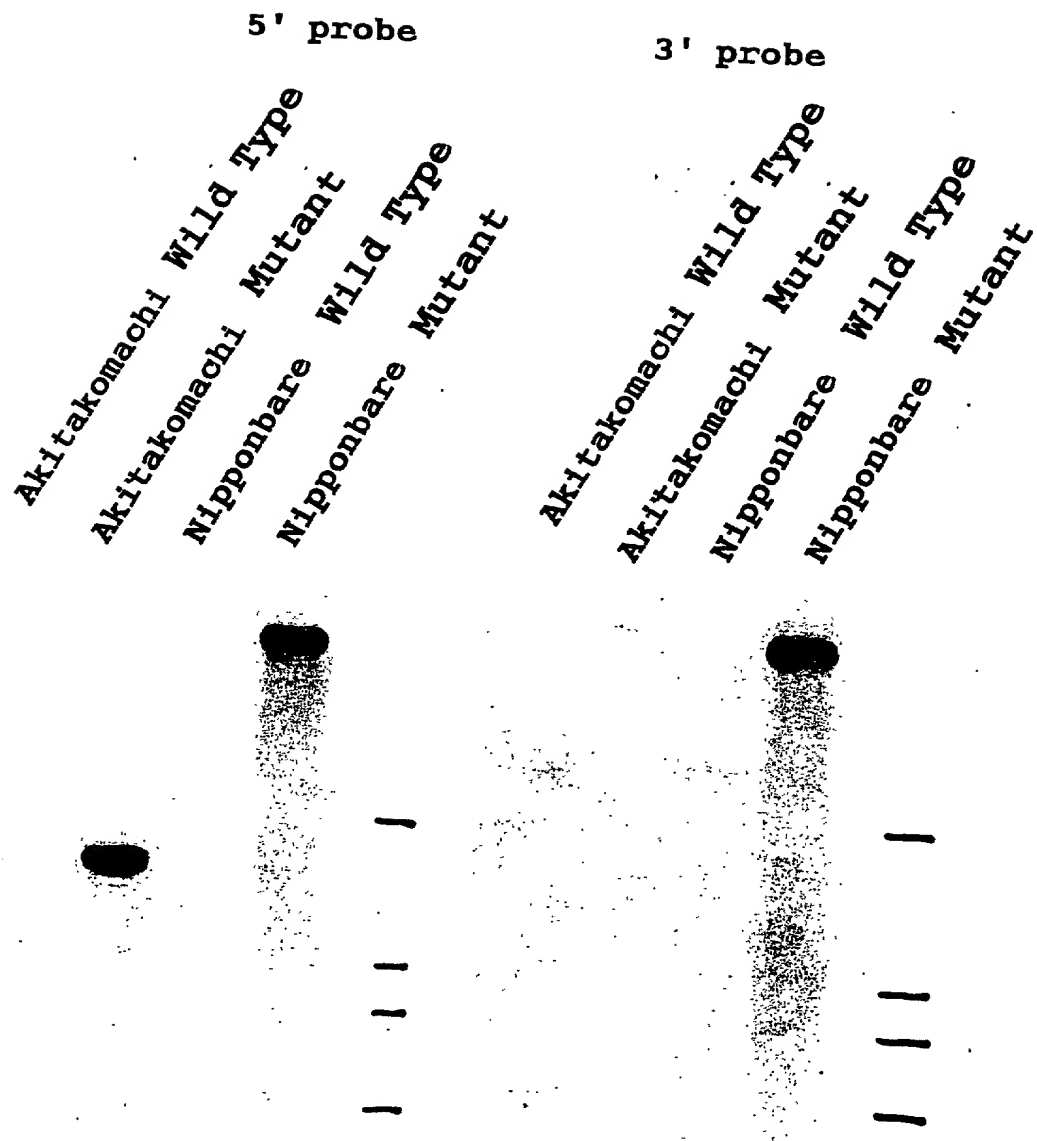


Fig. 3B

MEIVAVDQEG ARVVGTCNML ARGGTGAVAP VLELTATPRQ DAAAEAGVDE 50
 PAQHQCCEHFS IRGYVALLQK KDPKFCSLSR IFHDQKKCDE HKASSSPFSV 100
 AKFRRWDCSK CLDKLKTSDN GTAPRTLPAK QNGTSDGCSI TFVRSTFVPA 150
 SVGSQKVSPS TQSSQGNAD RSTLPKSVQE GNDKCNAPS GKNGAAEANT 200
 DSPMKDLQGP AQNYDVAANV SEDNTSVDVG ALPEVPQITW HIEVNGADQP 250
 PSTPKLSEVV LKRNEDENGK TEETLVAEQ NLTKDPNPMs GKERDQVAEQ 300
 CNLT KD PKFV SGQKCEQICN EPCEEVLEK SSKSKRKTDK KLMKKQOHSK 350
 KRTAQADVSD AKLCRRKPKK VRLLEIINA NQVEDSRSD E VHRENAADPC 400
 EDDRSTIPVP MEVSMDIPVS NHTVGEDGLK SSKNKT KRY SDVDDGSSL 450
 MNWLNKKKKR TGSVHETVAN PAGNLSNKKV TPTASTQHDD ENDTENGLDT 500
 NMHKTDVCQH VSEISTQRCS SKGKTAGLSK GAVHSAASTK YGGESTRNGQ 550
 NIHVLSAEDQ CQMETENSVL SHSAKVSPAE HDIQIMSDLH EQSLPKKKKK 600
 QKLEVTREKQ TMIDDIPMDI VELLAKNQE RQIMTENDCS DINRIQSKTT 650
 ADDDCVIVAA KGDSDYASSV FDTNSQQKSL ASQSTQKELQ GHLALTQES 700
 PHPQNFQSTQ EQQTHLRMEE MVTIAASSPL FSHHDDQYIA EAPTEHWGRK 750
 DAKKLTWEQF KATTRNSPAA TCGAQFRPGI QAVDLTSTHV MGSSSNYASR 800
 QPVIAPLDRY AERAVNQVHA RNFPSTIATM EASKLCDRRN AGQVVLYPKE 850
 SMPATHLLRM MDPSTLASFP NYGTSSRNQM ESQIHNSQYA HNQYKGSTST 900
 SYGSNLNGKI PLTFEDLSRH QLHDLHRPLR PHPRVGVLGS LLQKEIANWS 950
 ENCGTQSGYK LGVSTGITSH QMNRKEHFEA LNSGMFSAKW NALQLGSVSS 1000
 SADFLSARNS IAQSWTRGKG KMHVPLDRFV RQDICITNKN PADFTTISND 1050
 NEYMDYR 1057

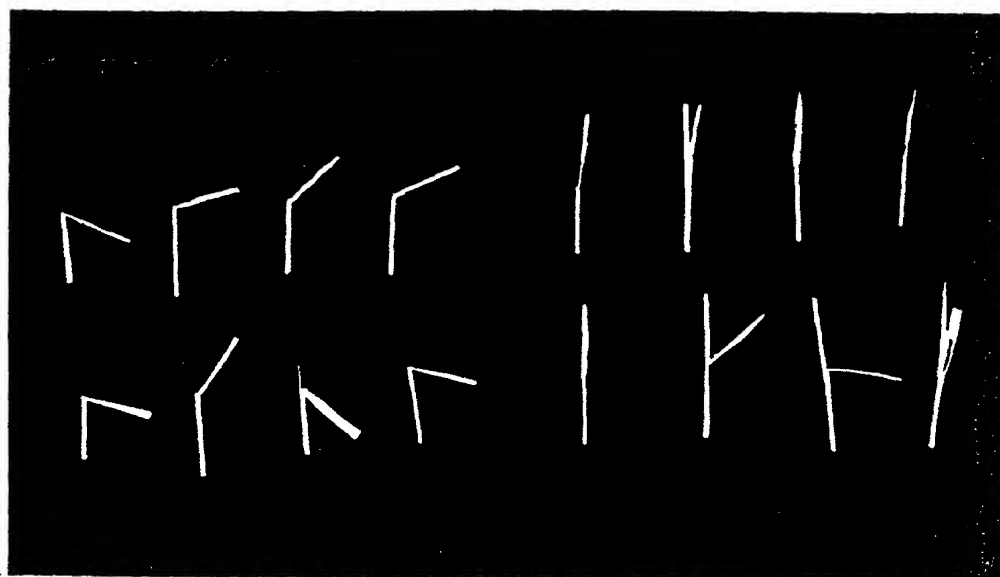
Nuclear localization
signal (1)

Nuclear localization
signal (2)

ATP/GTP binding motif

Fig. 4

Mutated line (A0369) derived from Akitakomachi

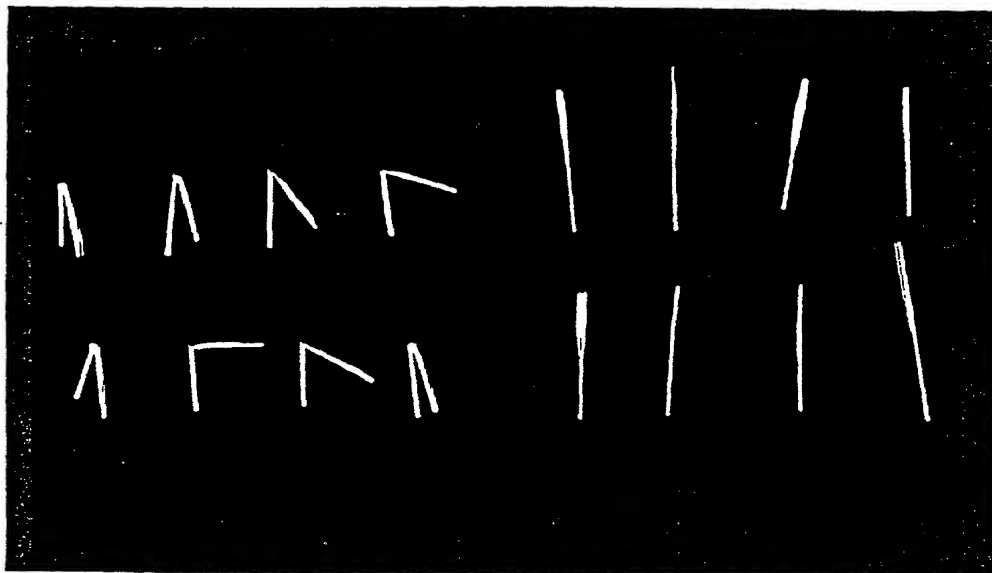


Wild Type

Mutant

Fig. 5A

Mutated line (NC6148) derived from Nipponbare



Wild Type

Mutant

Fig. 5B

DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

A NOVEL GENE INVOLVED IN BRASSINOSTEROID RESPONSES
the specification of which

(check ☒ [X] is attached hereto
one)

[] was filed on _____ as
Application Serial No. _____
and was amended on' _____.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I do not know and do not believe that the invention was ever patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application.

I do not know and do not believe that the invention was in public use or on sale in the United States of America more than one year prior to this application.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known by me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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